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Method Article

Air-drying of cells enables visualization of antiparallel microtubule overlaps in the spindle midzone

Aya Ifuji, Takahisa Kuga, Yuji Nakayama*

Department of Biochemistry & Molecular Biology, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan

A B S T R A C T

Immunofluorescence staining is used extensively to examine various types of cellular events. However, even when an antibody can detect its epitopes in western blotting, it sometimes fails to detect its epitopes when used for immunofluorescence staining. One example is the antiparallel microtubule overlaps in the anaphase and telophase spindle midzone, which functions as a signaling scaffold for cleavage furrow specification. It has been believed that it cannot be visualized by immunofluorescence staining due to the highly dense structure of microtubule overlaps (Ifuji et al., 2017). Here, we show a simple method for visualization of antiparallel microtubule overlaps in the anaphase and telophase spindle midzone.

- Air-drying cells before fixation enables visualization of antiparallel microtubule overlaps in the anaphase and telophase spindle midzone, which cannot be visualized by the conventional method.
- Simple method that requires minimal usage of equipment.
- Commonly used anti-tubulin antibodies can be used in this method.

A R T I C L E I N F O

Method name: Air-drying method

Keywords: Anaphase, Antiparallel microtubule overlap, Immunofluorescence staining, Microtubule, Midzone, Mitosis, Telophase

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Specifications Table

Subject area	• Biochemistry, Genetics, and Molecular Biology
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* Corresponding author.
E-mail address: nakayama@mb.kyoto-phu.ac.jp (Y. Nakayama).

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Method name	Air-drying method
Name and reference of original method	
Resource availability	

Background

Immunofluorescence staining is used extensively to examine various types of cellular events related to the sub-cellular localization of proteins, shape of organelles, cell cycle stage, activation of proteins, protein-protein interactions, and so on. Antibodies with high affinity to their epitopes are useful for this purpose; however, this technique does not always achieve the intended goal. Even when an antibody can detect its epitopes in western blotting, it sometimes fails to detect its epitopes when used for immunofluorescence staining, possibly through structural issues.

Microtubules play important roles in various cellular situations, including in mitotic cells, where the polymerization and depolymerization dynamics of microtubules are essential for their function [2]. Their dynamics and stabilities depend on microtubule populations. It is difficult to examine their differences by western blotting using total α- or β-tubulin as a marker, since all populations of microtubules are combined into a single lysate. Tubulin post-translational modifications have been reported; they include phosphorylation, polyglutamylation, acetylation, tyrosination and detyrosination [3]. If posttranslational modifications that represent microtubule populations would be identified, it may be possible to have quantitative data by western blotting. If not, immunofluorescence staining of cells enables the examination of the differences in dynamics and stabilities of microtubules. For example, stable microtubules can be detected by immunofluorescence staining after cold treatment, which is known to disrupt unstable microtubules [4].

The anaphase and telophase spindle midzone includes antiparallel microtubule overlaps and functions as a signaling scaffold for cleavage furrow specification [5]. Thus, investigation of the formation and regulation of antiparallel microtubule overlaps in the anaphase and telophase midzone provides insights into the regulation of cytokinesis. Since posttranslational modifications that represent the antiparallel microtubule overlaps have never been identified, it may be impossible to have quantitative data by western blotting. In addition, it is hard to synchronize all cells in anaphase and telophase, which is required for preparation of the lysate. We previously developed the method to achieve anaphase and telophase-enriched populations by brief treatment of cells with the lower concentration of nocodazole and the myosin II inhibitor blebbistatin [6]. However, less than 50% of cells could be synchronized in anaphase and telophase. Thus, immunofluorescence staining is useful to analyze antiparallel microtubule overlaps in the anaphase and telophase midzone. Antiparallel microtubule overlaps have a highly dense structure; therefore, it has been believed that they cannot be visualized by immunofluorescence staining [1,7,8].

Here, we show a simple and fast method for visualization of antiparallel microtubule overlaps, which is not visualized by the conventional staining method, in the anaphase and telophase midzone with commonly used antibodies.

Method details

To visualize antiparallel microtubule overlaps in the anaphase and telophase midzone, air-dry cultured cells and then fix these cells with PTEMF buffer (2 mM PIPES [pH 6.8], 0.2% Triton X-100, 10 mM EGTA, 1 mM $MgCl_2$, 4% formaldehyde) [9]. The fixed cells can be subjected to standard immunofluorescence staining with commonly used anti-tubulin antibodies. The protocol is as follows:

1) Prepare the equipment as shown in Fig. 1a. Stick two pieces of double-sided tape for two dishes to a board made of expanded polystyrene, which is used to prevent cells from cooling (Fig. 1b). Place a hair dryer fixed in a stand at approximately 7.5 cm above the expanded polystyrene board (Fig. 1g). The height of a hair dryer should be adjusted on the basis of strength of air flow.